# Synthesis and anticancer potential of combretastatin sulfonamide/sulfonates, quinazolinone derivatives and green synthesis of spirooxindole, pyrazolo-pyrimidine derivatives

ABSTRACT SUBMITTED TO

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**BY**

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# SYNOPSIS

The work carried in the research tenure has been compiled in the form of a thesis entitled ‘‘***Synthesis and anticancer potential of combretastatin sulfonamide/sulfonate, quinazolinonederivatives and green synthesis of spirooxindole, phyrazolo-pyrimidine derivatives***”. The main aim of this work is the design and synthesis of new conjugates of biologically active moieties combretastatin sulfonamide/sulfonate, quinazolinonederivatives, spirooxindole, phyrazolo-pyrimidine derivatives and their evaluation of cytotoxic activity as well as catalyst free green synthesis of spirooxindole, phyrazolo-pyrimidine derivatives.Further objective was to study the mechanism of action of these new active conjugates with particular focus on the inhibition of tubulin polymerization and the thesis has been divided into four chapters.

- **CHAPTER I:** This chapter provides a general introduction about chemotherapeutic agents, particularly tubulin polymerization inhibitors such as colchicine, combretastatin, phenstatin, aryl oxindoles and benzene sulfonamides.

- **CHAPTER II:** This chapter describes the synthesis, *in-vitro and in-vivo*evaluation of novel (*Z)*-3,4,5-trimethoxystyryl benzenesulfonamide/sulfonate derivatives as highly potent tubulin polymerization inhibitors.The potent conjugates from this class were further investigated fortubulinpolymerization, microtubule network perturbation and bioavailability studies.

- **CHAPTER III(SECTION-A):** This chapter illustrates design, synthesis and biological evaluation of quinazolinone-urea as potent antiproliferative agents. Further, these compounds have been evaluated for their cytotoxicity, inhibition of tubulin polymerization and apoptosis.

- **CHAPTER III (SECTION-B):** This chapter illustrates synthesis and biological evaluation of quinazolinone- arylpropenonesderivatives as potential anticancer agents. Further, these compounds have been evaluated for their cytotoxicity.

- **CHAPTER IV (SECTION-A):** This section deals with the green synthesis and biological evaluation of new spirooxindoleconjugates as potent anticancer agents. Further, these compounds have been evaluated for their antimitotic activity.

- **CHAPTER IV (SECTION-B):**This sectiondescribes the green synthesis and biological evaluation ofphyrazolo-pyrimidine congeners, mostly focused on the synthesis by environmental friendly methods.

## CHAPTER-I

**Introduction**

Cancer, characterized by uncontrolled growth or spread of abnormal cells, poses a significant challenge with high rates of diagnosis and mortality. In the treatment of cancer, chemotherapy plays a very important role in solid tumors and is used as an adjuvant to initial therapy by surgical or radio therapeutic procedures. Chemotherapy becomes critical to effective treatment because only systemic therapy can attack micro metastases. The chemotherapeutic agents target the fast dividing abnormal cells and can be categorized into functional sub groups: alkylating agents, antimetabolites, antibiotics, and antimitotics such as microtubule targeting agents.

Microtubules are protein biopolymers formed through polymerization of heterodimers of $\alpha$- and $\beta$-tubulin. Tubulin polymerization is reversible, and the dynamic assembly and disassembly of microtubules are involved in a number of cell functions, including cell division, migration, and shape change. Many natural and synthetic compounds are reported to target the tubulin−microtubule system.Antimitotic agents derived from natural or synthetic products generally exert their effect as microtubule stabilizers or polymerizing agents, like taxol paclitaxel (**Ia**) and docetaxal (**Ib**), which block the microtubule disassembly. They bind at the $\beta$-tubulin site in the microtubules and are used in the treatment of carcinomas, like lung, breast, ovarian and bladder. In contrast, microtubule destabilizers such as colchicine, vinca alkaloids, combretastatin A-4 and E7010 (**V**) bind at the $\beta$-tubulin site in microtubules and cause the depolymerization of microtubules. Many of such agents manifest different limitations in their clinicalutility,therefore development of new microtubule targeting agents is of significance.

Combretastatin A-4 (**IVa**), is another excellent tubulin polymerization inhibitor that binds to the colchicine binding site of the tubulin and demonstrates cytotoxicity against a broad spectrum of human cancer cell lines including MDR cancer cells. However, the *in vivo* efficiency of CA-4 is limited due to its poor pharmacokinetics resulting from its high lipophilicity and low water solubility. The structural modifications on CA-4 has led to the development of a number of new CA-4 derivatives that exhibit potent tubulin polymerization inhibitors such as combretastatin A-4 phosphate (**IVb -** CA4P, Figure 1A) and CA-1 disodium phosphate (CA1P- Oxi 4503) as prodrugs.Similarly, amino substituted combretastatinderivative(**IVc**- AVE-8063) and serine prodrug of combretastatin amine (**IVd**- Ombrabulin) have been developed. These prodrugs of CA-4 are in advanced stages of clinical studies.

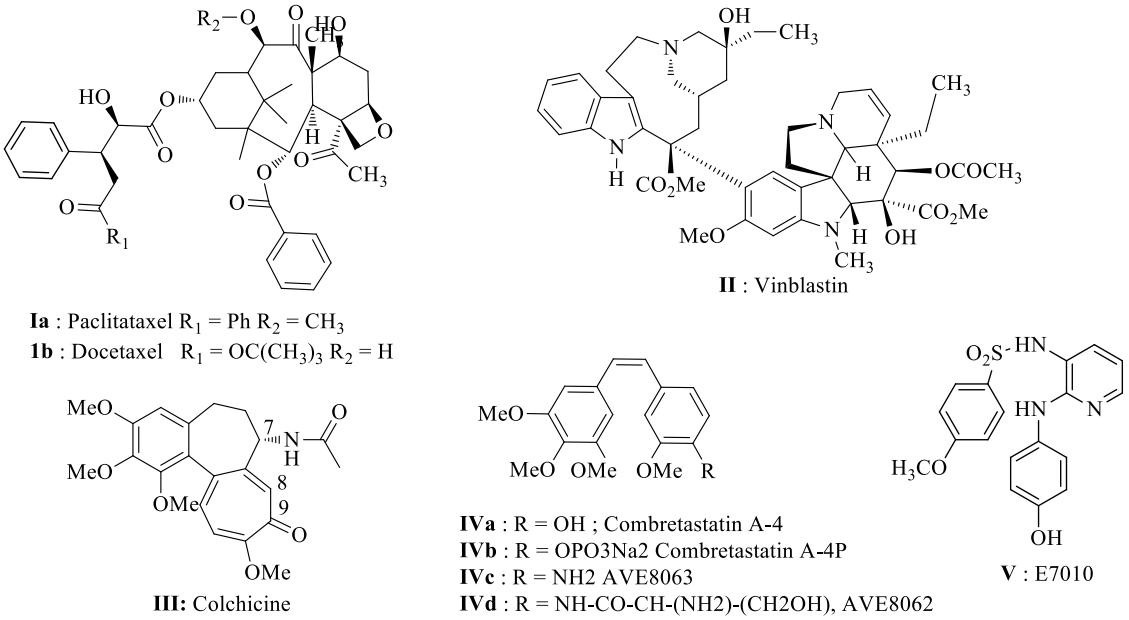



**Figure 1.**Chemical structure of microtubule targeting agents

# CHAPTER-II

## Synthesis, *in-vitro* and *in-vivo* Evaluation of (*Z*)-3,4,5-Trimethoxystyryl Benzenesulfonamide/sulfonate Derivatives as Highly Potent Tubulin Polymerization Inhibitors

Combretastatin A-4 (CA-4) exhibits potent cytotoxicity with a broad spectrum of antitumor activity. CA-4 and related derivatives are microtubule inhibitors which target the colchicine binding site and exhibit low toxicity profiles. Despite its potent cytotoxic and antitubulin activities, CA-4 failed to exhibit potent antitumor efficacy in animal models due to its limited water solubility (poor biosolubility). Because of its interesting antitumor activity and simplicity of structure, a number of CA-4 analogues (**IVa-d**) have been synthesized with modifications to ring A, ring B, and the bridge. In addition, the SAR studies of CA-4 analogs have shown that, both hydroxy and amino substituents contribute to improve anti-mitotic activity and provide the opportunity to structurally modify this scaffold. Particularly, the changing positions of amino group on combretastatins exhibits vary cytotoxicity and anti tubulin activity. The sulfonamide moiety is easy to install as well as it generally imparts stability and crystallinity to the attached pharmacophore.The beneficial influence of sulfonamide substituents on the anticancer potential of designed compounds have been established earlier. The antitubulin agents such as E7010 (**V**),E7070 (**VI**, indisulam) and the biarylsulfonamide (**VII**) constitute a few illustrative examples that are pertinent to the present work. Thus, it is clear from the back ground presented above, that the pharmacophores representedby combretastatin and sulfonamide offer excellent possibilities for hybridization towards the design of improved tubulin polymerization inhibitors. Keeping this in mind, we linked the amino and hydroxyl substituted combretastatin derivatives with sulphonamides to generate combretastatin-sulfonamide hybrids.

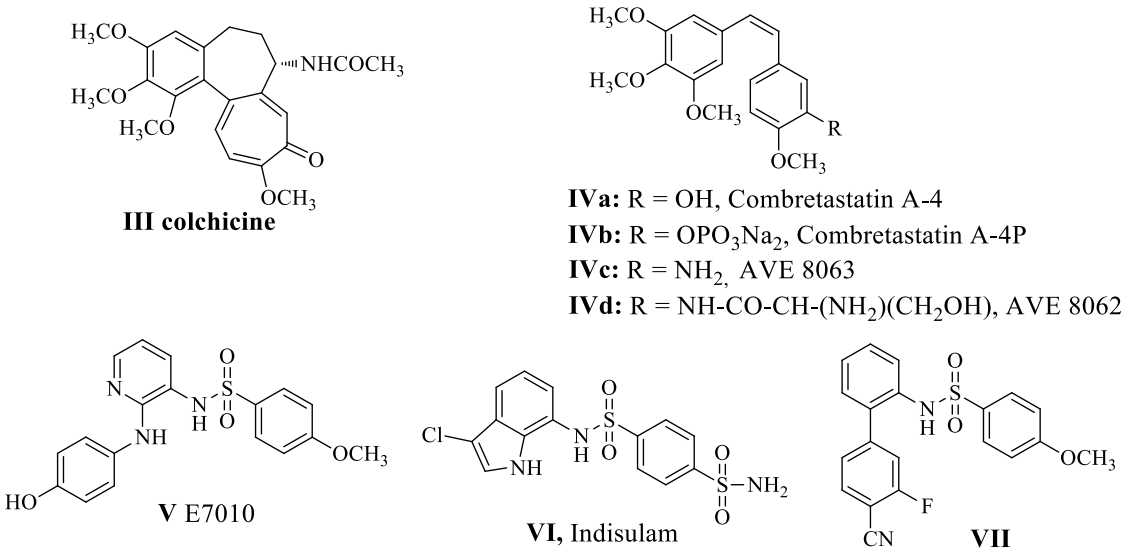
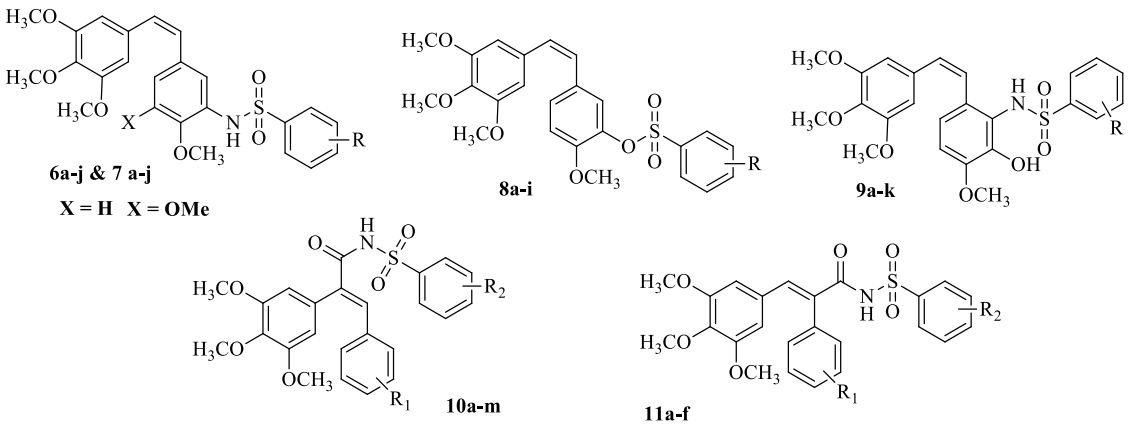



**Figure 2.** Chemical structure of microtubule targeting agents: colchicines (**1**) CA-4 (**2a**), CA-4P (**2b**), AVE8063 (**2c**), AVE8062 (**2d**) and synthetic benzensulfonamide derivatives.

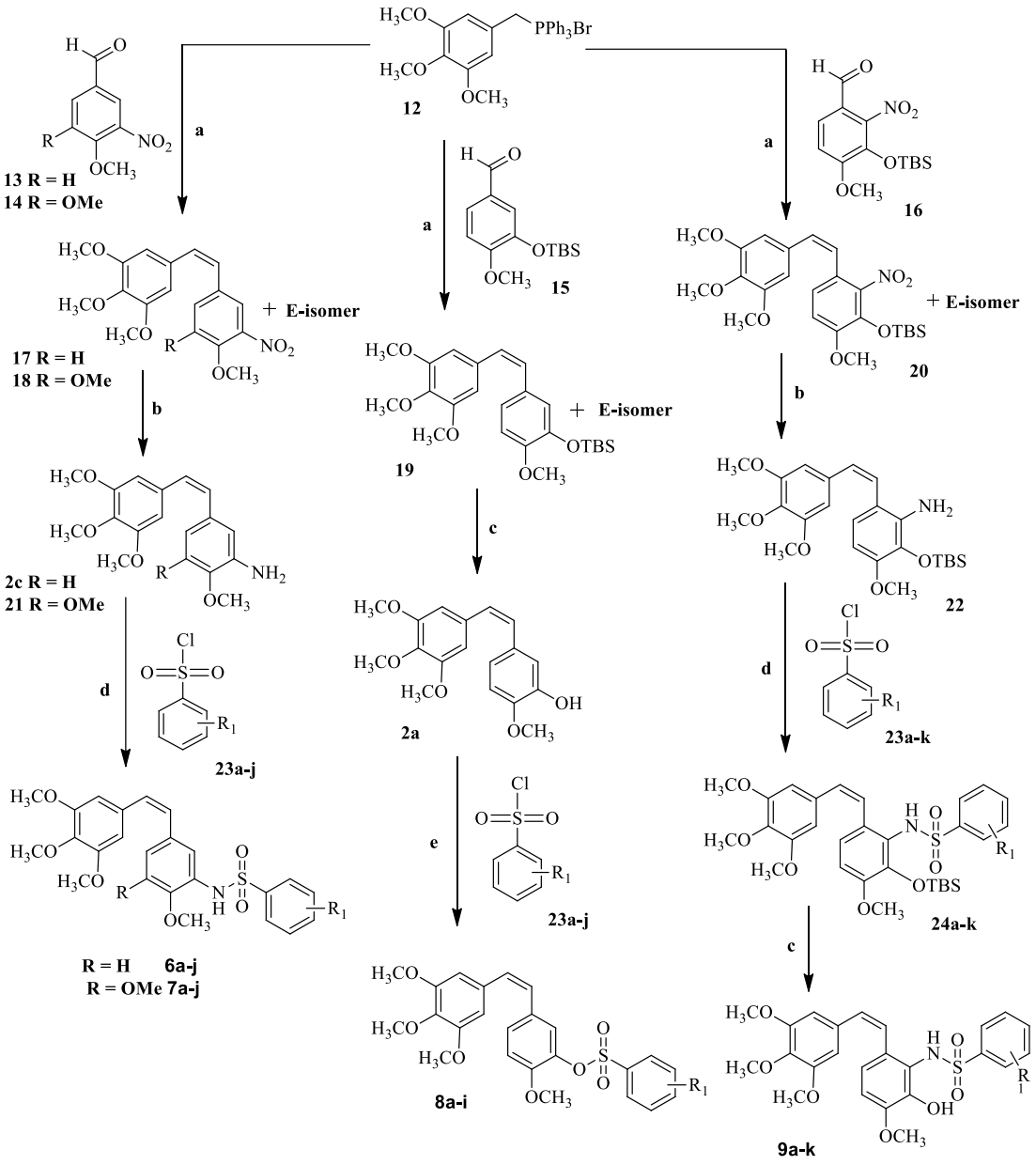
## Designed molecules



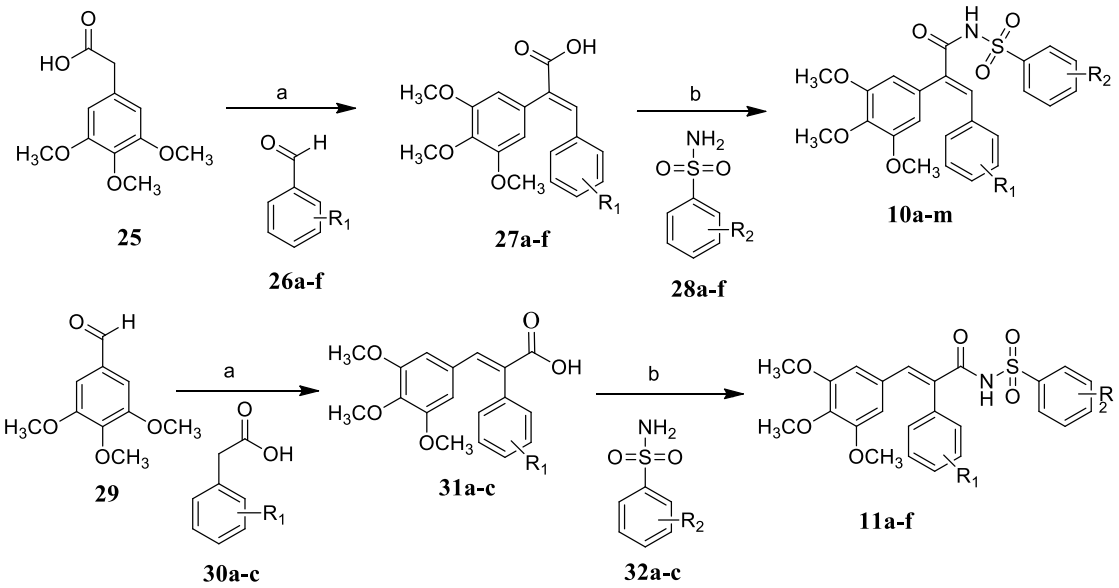

## Chemistry

Compounds **6a-j,7a**-i and **9a**-k were prepared by coupling of properly substituted (Z)-(3,4,5-trimethoxystyryl)anilines (**2c, 21** and **22)** with various commercially available substituted benzenesulfonylchlorides (**23a-k**) in a 1:4 (v/v) mixture of pyridine in anhydrous dichloromethane13 and (Z)-3-styrylphenyl benzenesulfonates (**8a-i**) prepared by the reaction of combretastatin (**2a**) with the similar benzenesulfonyl chlorides by using dichloromethane as a solvent in the presence of triethylamine with outstanding yields. The intermediate compounds **2a, 2c, 21 and 22** were obtained by the reduction and deprotection of their respective nitro, hydroxyl combretastatin derivatives (**17**-**20**), which were synthesized by double bond

forming Wittig reaction between the suitable benzaldehydes (**13-16**) with 3,4,5-trimethoxybenzyl triphenylphosphonium bromide (**12**) in presence of sodium hydride in anhydrous dichloromethane, afforded a mixture (1:1, v/v) of nitro Z-stilbenes (**17-20**) and E-stilbenes as depicted in Scheme 1 and these isomers have been separated by column chromatography. The compounds (**10a-m** and **11a-f**) have been achieved by the formation of amide bond between the stilbene-acrylic acids (2**7a**-f and **31a-c**) with a variety of substituted benzene sulfonamides (**28a-f** and **32a-c**) by using EDCI/DMAP. Wherein, the intermediates were prepared in good yield by the Claisen–Schmidt condensation of different phenyl acetic acids with appropriate benzaldehydes.



**Scheme 1.**Reagents and conditions: (a) NaH, dry $CH_2Cl_2$, 0 °C to rt, 12 h; (b) Zn, $HCO_2NH_4$, MeOH, rt, 4 h; (c) TBAF,THF 0 °C, 2 h (d) Pyridine, 0-5 °C, 4 h; (e) TEA, $CH_2Cl_2$, 0-5 °C, 4 h.




**Scheme 2 & 3.***Reagents and conditions:(*a) $AC_2O$, NaOH, 140 °C; (b) EDCI, DMAP, TEA, $CH_2Cl_2$.

**Biological studies**

The synthesized derivatives (Z)-N-(3-styrylphenyl)benzenesulfonamides/sulfonates (**6a-j, 7a-i, 8a-i, 9a-k, 10a-m** and **11a-f**) have been evaluated for their cytotoxicity against a panel of sixty human tumor cell lines of National cancer institute (NCI). Over sixty compounds, eleven compounds were evaluated in the secondary screening at five dose concentrations with 10-fold dilutions which displayed remarkable antiproliferative efficacy on most of the NCI cell lines. Mostly B-ring linked sulphonamides (**6a-j, 7a-i, 8a-i**and **9a-k**) exhibited remarkable antiproliferative activity. Specifically, the compound **7a** and **9a** possess broad spectrum of cytotoxicity within nanomolar range against most of cell lines with GI50 values below 0.03 µM, wherein, relatively twenty five cell lines showed $GI_{50}$ values less than 0.05 µM.

The SAR studies have been examined for these diverse combrestatin sulfonamide analogs. In particular, the aryl sulphanamide has been attached on B-ring and *cis*- double bond. Mostly B-ring linked sulphonamides (**6a-j, 7a-i, 8a-i** and **9a-k**) exhibited remarkable antiproliferative activity as compared to the double bond linked sulfonamides (**10a-m** and**11a-f**).

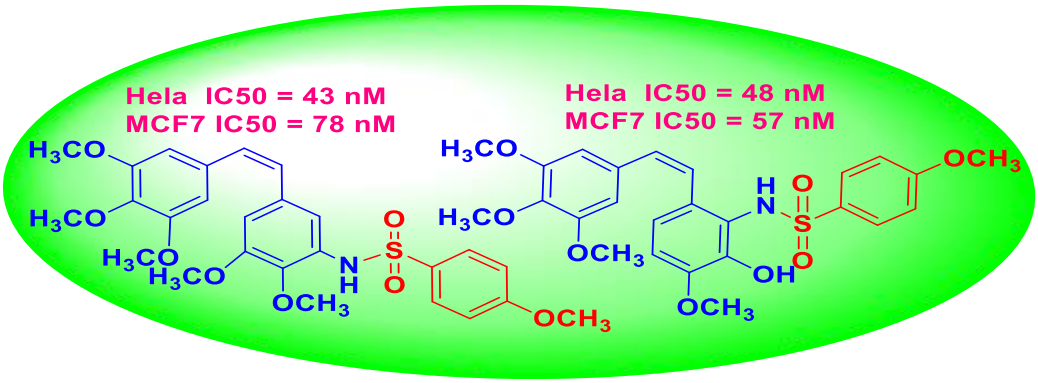



**Figure.3**Most active compounds cytotoxicity values

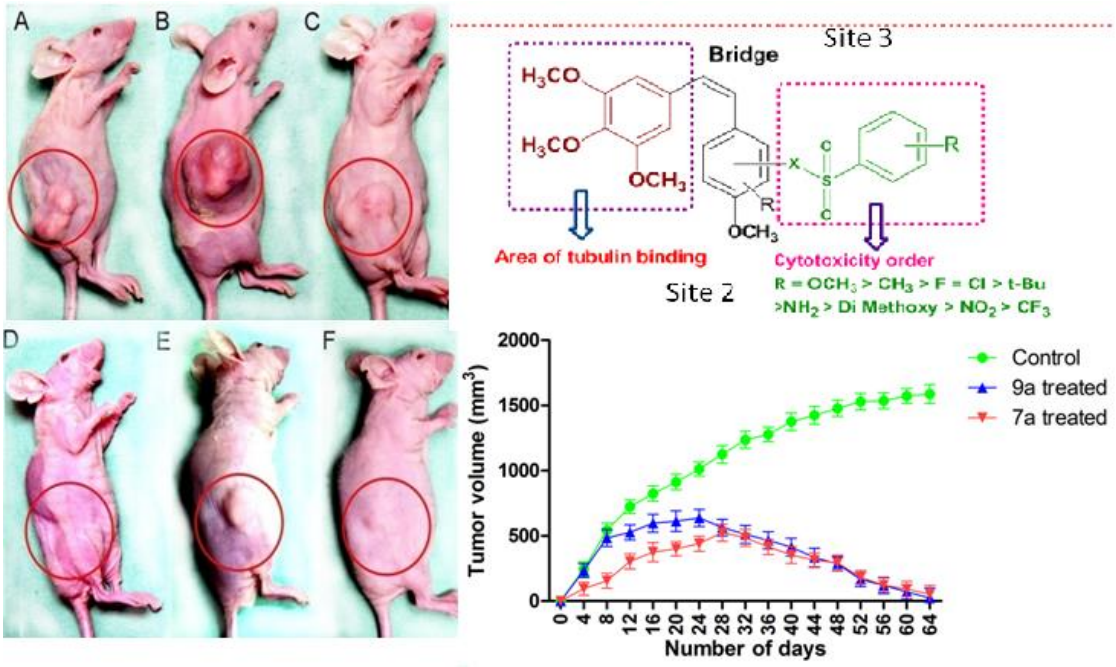



**Figure.4** Structure–activity relationship (SAR) and in vivo human cervical cancer HeLa xenograft model.Figure A, C and E represent nude mice after tumor induction. Figure D and F represent the mice after the treatment with **7a** and **9a** for 60 days respectively. Similarly, the figure B, represent the mice to which neither **7a** nor **9a** was given (vehicle control) during the same period.

## Summary

In summary, we have presented data that supports the potential for two molecules **7a** and **9a** with methoxy group at *para* position of C-ring of the trimethoxystyrylbenzenesulfonamide/sulfonates scaffold as novel anticancer agents. All the evidences gathered suggest that these trimethoxystyrylbenzenesulfonamide/

sulfonate analogues exert promising antiproliferative by effectively inhibiting the tubulin polymerization and disrupt the microtubule dynamics in cancer cells. Compounds **7a** and **9a** also exhibited promising efficacy in HeLatumorxenograft model and have shown an excellent therapeutic window and we believe that these molecules have significant potential for clinical development as new anticancer agents.

***Chem. Med. Chem.*2017,** *9*, 678-700**.**

## CHAPTER-III (Section-A)

**Design, Synthesis andBiological Evaluation of Quinazolinone-Urea Derivatives as Potential Anticancer Agents**

In continuation of our interest in the design and development of new anticancer agents, by simple heteroaromatic organic compounds like quinazolinone that possess promising cytotoxic activity. Recently we reported that quinazolinone-chalconesderivatives and Urea derivatives that were exhibitedpromising cytotoxic activity. Quinazolinones are important due to their wide range of biological activities such as antitumor, anti-angiogenesis and anti vascular activity. Some of the quinazolinonepharmacophores like HMJ-38 (**VIII**)is reported as the most potent 2-phenyl-4-quinozolinone derivatives (**IX**to**XII**) in inhibiting the tubulin polymerization and showed significant cytotoxicity against several human tumor cell lines.

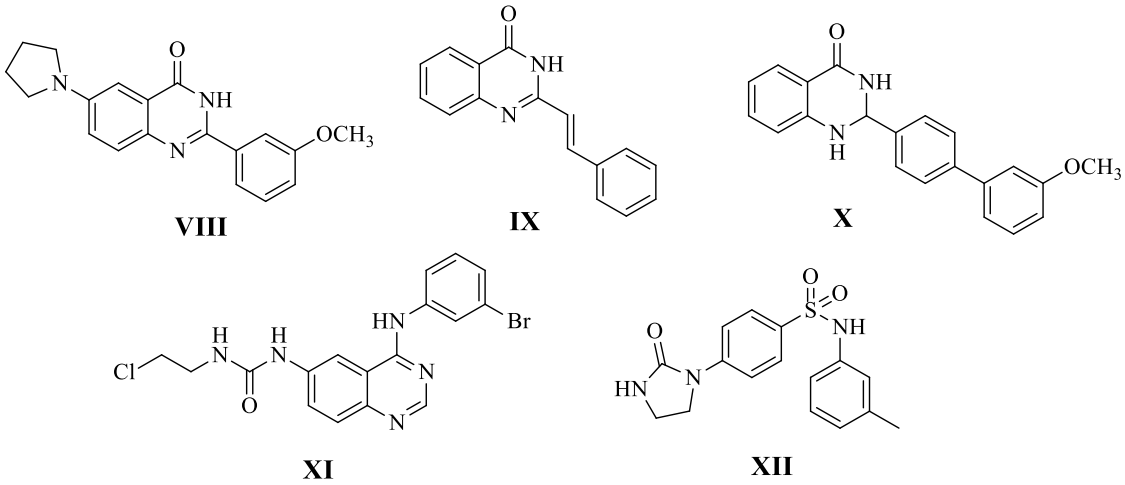
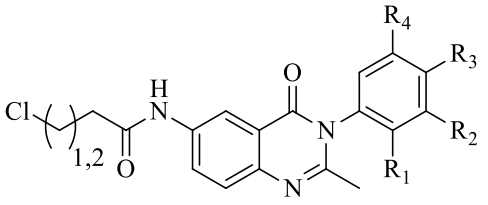
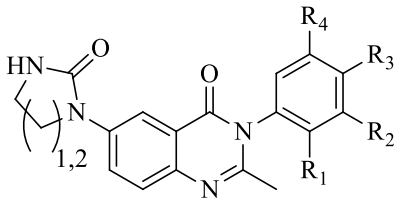



**Figure 5:** Chemical structures ofbiologically active quinzolinone derivatives and tubulinpolymerization inhibitors.

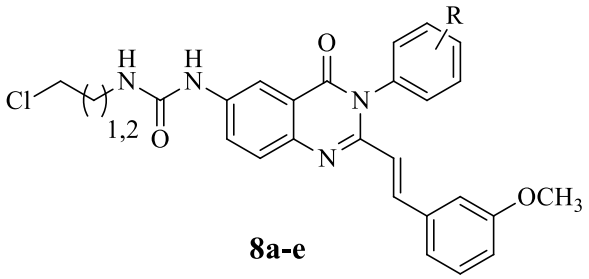
By collecting the above facts, we have synthesized a series of quinazolinone urea derivatives (**6a-j, 7a-j, 8a-e,** and **9a-e**) and tested for their cytotoxic activity against various human cancer cell lines. Compounds **7a** and**7c** exhibited significant antiproliferative activity against all most of the cell lines, with IC50 value of < 1 µM. All synthesized compounds (**6a-j, 7a-j, 8a-e,** and **9a-e)**displayed cytotoxicity against A549 (non-small cell lung cancer), HeLa (cervical carcinoma), MCF-7 (breast cancer) and B16 (colon carcinoma) with IC50 values ranging from 0.65-5.60µM. Cell cycle assay revealed that these compounds arrested the G2/M phase of the cell cycle.Flowcytometric analysis revealed that these conjugates arrested the cell cycle at the $G_2$/M phase. In addition, compounds **7a**and **7c**exhibited inhibitory effect on the tubulin assembly with an $IC_{50}$ value of **0.65**µM and **0.85**µM respectively. Moreover, Hoechst staining, and activation of caspase-3 suggested that these conjugates (**7a**and**7c**) induce cell death by apoptosis. Molecular docking studies demonstrate these conjugates effectively bind with the colchine binding site of the tubulin. Overall, this investigation describes the synthesis of quinazolinone-ureaderivatives as potential anticancer agents with apoptosis inducing ability by targeting tubulin.



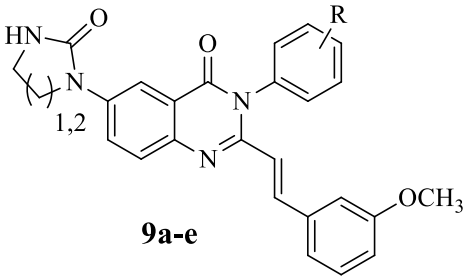

**6a-j**




**7a-j**




**8a-e**

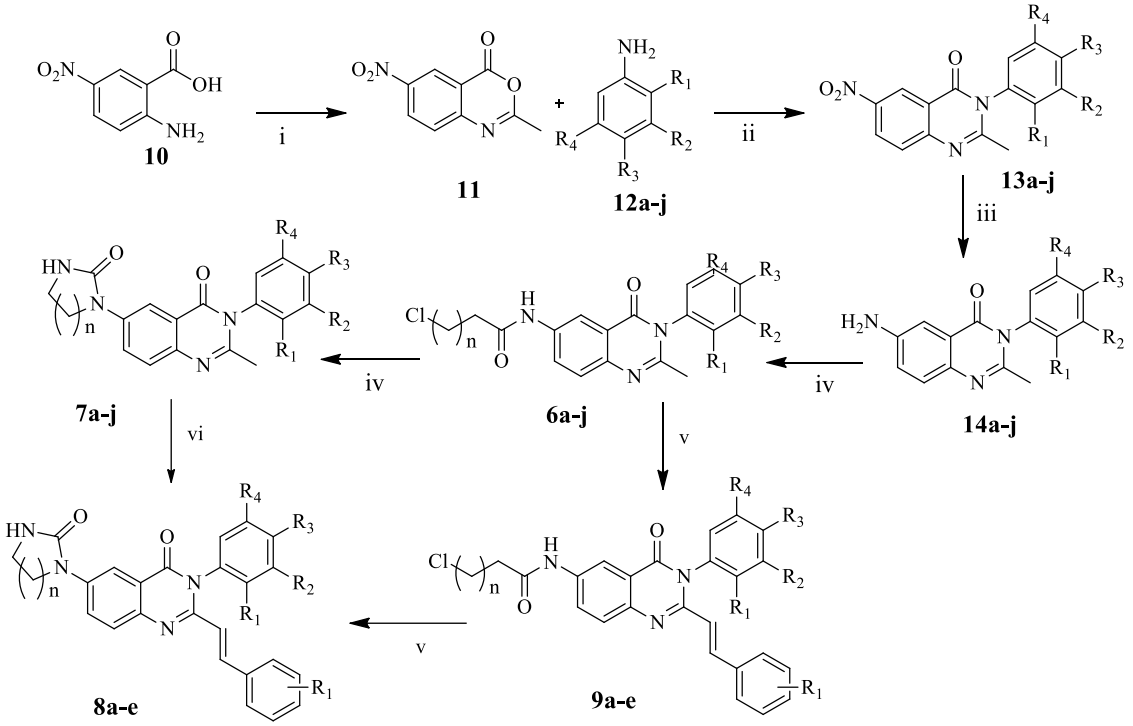


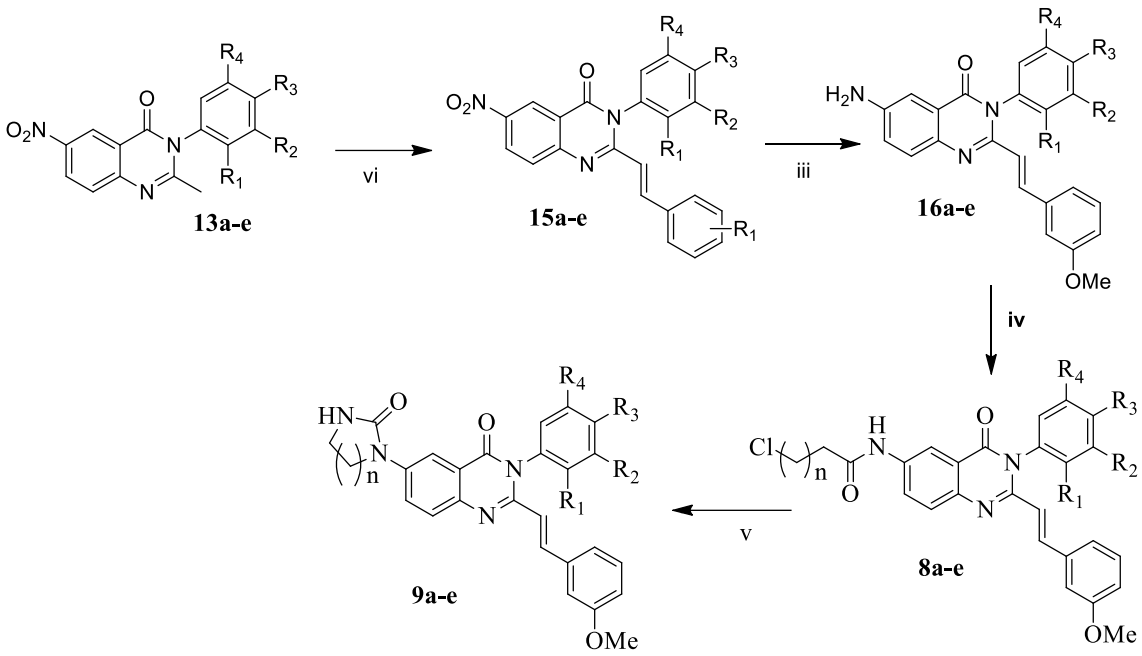

**9a-e**

**Chemistry.**

The cyclizedquinazoliinoneurea (**7a-j** and **9a-e**) derivatives were prepared by the cyclization of The 3-chloropropyl quinazoliinone urea and 2-chloroethyl

quinazoliinone ureaderivatives (**7a-j** and **9a-e**) respectivelyand these were prepared by the coupling of 3-chloropropyl isocyanide,2-chloroethyl isocyanide with amino-quinazolinones (**14a-j**), as shown in Scheme 4. Aminoquinazolinones were obtained by the reduction with the correspondingnitro-quinazolinones(**13a-e**) and the crucial intermediates such as compounds of these nitro-quinazolinones were obtained by reaction with substituted anilines (**12a-j**) with 5-nitro anthranilicacid**10**.






**Scheme 4& 5:** *Reagents and conditions:* (a) $AC_2O$, reflux, 1h, 95%; (b) ACOH, reflux, 3h, 90%; (c) Pd-C, $H_2$, EtOAC, 3h, 90%; (d) 2-Chloroethylisocyanate, $CH_2Cl_2$, 0 °C(e) Benzaldehydes, ACOH.

**Summary**

A series of quinazolinone urea derivatives (**6a-j, 7a-j, 8a-e**and **9a-e**) were designedsynthesized and evaluated for their cytotoxicity against different human cancer cell lines. Compounds **7a, 7c**and **9a** exhibited significant antiproliferative activity against all most of the cell lines, with IC50 value of < 1 µM.Most of the compounds that exhibit antitumor activity against various cancer cell lines as well as tubulin polymerization inhibition.

*(Manuscript prepared for **Chem. Med.Chem**)*

## CHAPTER-III (SECTION-B)

### Synthesis and Biological Evaluation of Quinazolinone-Arylpropenones Derivatives as Potential Anticancer Agents

Several quinazolinone derivatives were synthesized recently with an aim of finding related compounds having therapeutic efficacy with less side effects. In the process HMJ-38 is reported as the most potent 2-phenyl-4-quinozolinone derivative in inhibiting the tubulin polymerization (Fig. 6) and showed significant cytotoxicity against several human tumor cell lines. In recent years there has been an increasing

interest in the chemistry of quinazolinones because of their wide range of biological significance.

2-Styryl quinazolinone (**XIII**) derivatives form an important component of pharmacologically active compounds because they are associated with inhibitory effects on tubulin polymerization, as shown in Figure. Poly(ADP-ribose) polymerase (PARP) is anabundant nuclear enzyme which is involved in a number of cellular processes like DNA repair and programmed cell death. Indole derivatives(**2**) and (**3**) is known to inhibit this DNA repair enzyme poly(ADP-ribose) polymerase (PARP) Therapeutic agents containing the quinazolinone core structure are in the clinic and as well undergoing clinical trials for the treatment of cancer. And Reddy group in (2012) reported a series of(Z)-arylaminopropenones (**XVI**) and (**XVII**) molecules, which induce apoptotic death of a wide variety of human tumor celllines at nanomolar concentrations by promoting Anti cancer activity.

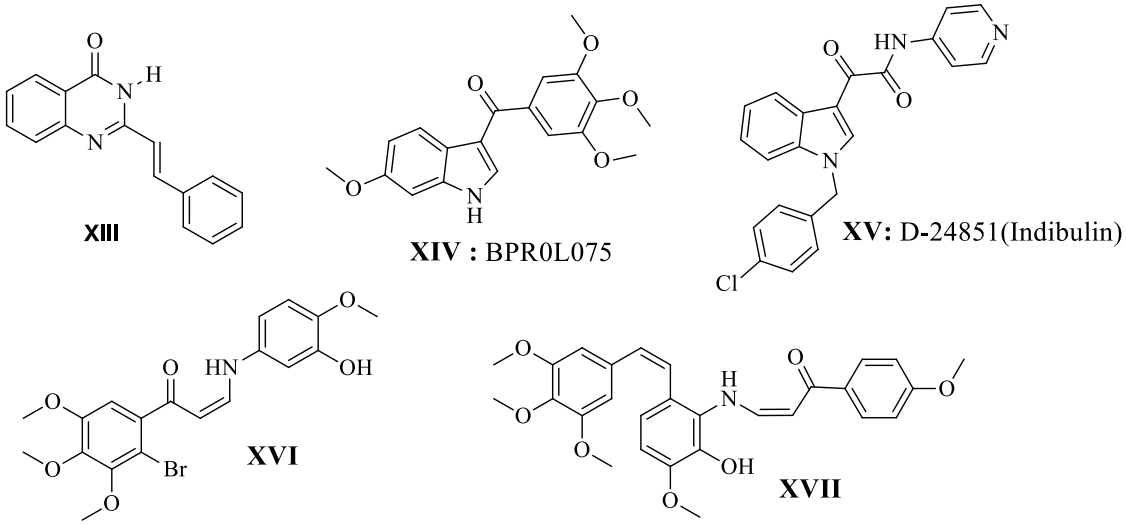
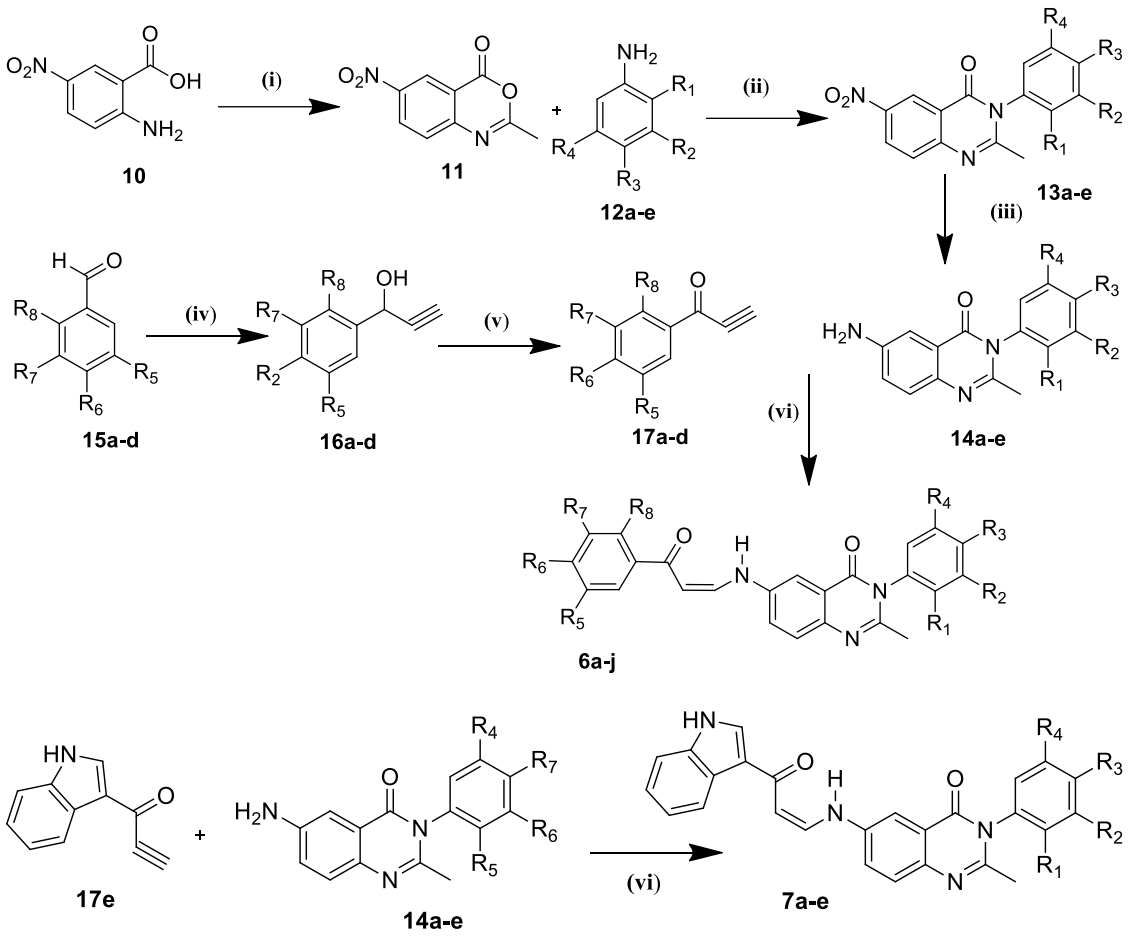



**Figure 6:** Chemical structures ofbiologically active quinazolinone, indole and arylpropenone derivatives

**Chemistry**

Aryl (phenylamino) propenonequinazolinone (**6a-j**and **7a-e**) derivatives were prepared by thephenylquinazolinonederivatives(**8a-e)**, with the arylpropenone derivatives in ethanol solvent. The reaction mixture was stirred at a temperature of 25-35 °C for 4 h and these amino-quinazolinones (**14a-e**), as shown in Scheme 6 were obtained by the reduction with the correspondingnitro-quinazolinones**(13a-e)**. The

crucial intermediates such as compounds of these nitro-quinazolinones were obtained by reaction with substituted anilines **(6a-j)** with 5-nitro anthranilicacid(**10)**.




**Scheme 5:** *Reagents & conditions:* (i) $(CH_3CO)_2O$, 150 $^oC$, 30 min.; (ii) Substituted anilines, AcOH, reflux, 4 h; (iii) Pd-C, $H_2$, EtOH, rt, 3 h.(iv)Ethynylmagnesium bromide, THF, 0 $^oC$, 2 h (v) IBX, DMSO, rt, 4 h, (vi)EtOH, rt, 3 h.

**Summary**

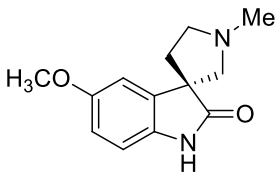
A series of (Z)-1-Aryl (phenylamino) propenonequinazolinone (**6a-j**and**7a-e**) were designed, synthesized and evaluated for their cytotoxicity against different human cancer cell lines. Compounds **6a** and **7a**exhibited significant antiproliferative activity against all most of the cell lines, with IC50 value of 1.23-3.73µM. Most of the compounds that exhibit antitumor activity against various cancer cell lines as well as tubulin polymerization inhibition.

*(Manuscript prepared for **Eur. J. Med. Chem**.)*

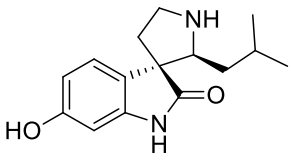
## CHAPTER-IV (SECTION-A)

**Discovery of PyrrolospirooxindoleDerivatives as DovelCyclinDependent kinase 4 (CDK4) Inhibitors by Catalyst-free, Green Approach**

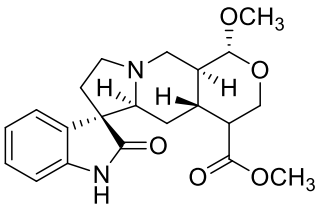
Spirocyclic compounds are notable for their molecular architecture as well as the prominent biological activities.Especially, spirocyclicoxindoles are attractive synthetic targets because of their widespread occurrence in various natural products and biologically active molecules. The structural rigidity imparted by the spiro-carbon causes conformational restrictions and this may influence the binding of such compounds to the biological targets favourably. A selected assortment of natural products incorporating the spirooxindole unit is depicted in Figure. Spirotryprostatin A and B (**XXIa**and **XXIb**) are two natural alkaloids isolated from the fermentation broth of *Aspergillus fumigates*, that have been identified as promising inhibitors of microtubule assembly(Fig. 7). Spirooxindole systems also constitute the core scaffold of many synthetic pharmaceuticals with a wide range of biological applications such as antimicrobial, antitumor, antibioticand inhibitors of the human NK-1 receptor.



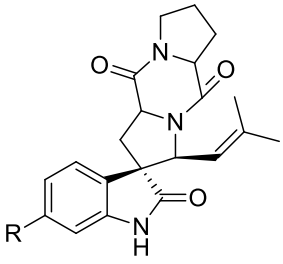
**XVIII** Horsifiline



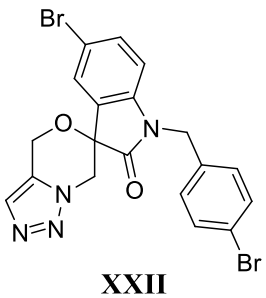
**XIX** Elacomine



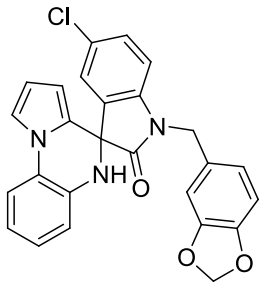
**XX** Pteropodine



**XXIa** R = $OCH_3$ Spirotryprostatin A
**XXIb** R = H Spirotryprostatin B



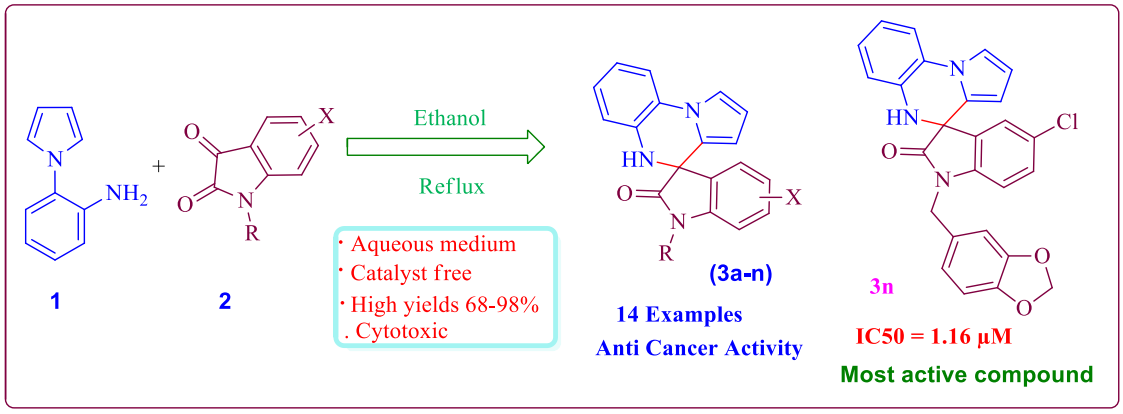
**XXII**



**9n**

**Figure7:** Chemical structures ofbiologically active spirooxindole derivatives and CDK4 inhibitors

In the course of our interest to develop nitrogen heterocyclic compounds as anticancer agents, prompted us to investigate the biological activity of spirooxindole scaffold in detail. This called for a convenient and practical method for the synthesis of the spirooxindole unit. Literature survey reveals that 1,3-dipolar cycloaddition reactions have been used extensively to prepare spirooxindole derivatives. The preparation of such compounds via the reaction of isatin and N-(2-aminophenyl)pyrrole at room temperature in the presence of different catalysts such as molecular iodine, $ZnCl_2$, $CuCl_2$, $FeCl_3$, p-TSA, Sulfamic acid and HCl are also reported. In many cases the desired products were obtained in very low to moderate yields. Our efforts led to the development of a catalyst-free synthesis of pyrrole fused spirooxindoles in ethanol (Scheme 6).




**Scheme 6:**Green synthesis of cytotoxic spirooxindole derivatives

In conclusion, a simple, mild, efficient and environmentally benign method for the synthesis of 5'H-spiro[indoline-3,4'-pyrrolo(1,2-a)quinoxalin]-2-ones has been developed without using a catalysts in ethanol. The advantages of this method include its simplicity of operation, cleaner reactions, absence of side products and higher yields. Further, the purification of the product is simple, involving a simple filtration and washing. The synthesized compounds are tested for their cytotoxocity and they exhibit $IC_{50}$ values in the range 31.44 - 1.16 µM. Compound **3n** with piperonyl substitution on 5-chloroisatin nitrogen displayed the highest cyctotoxicity in the series. Its $IC_{50}$ values are comparable to that of the positive control, doxorubicin. Further mechanistic studies such as cell cycle analysis, mitochondrial potential assay, annexin

V-FITC assay and Western blot analysis indicated that **3n** induces cell death by apoptosis.

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## CHAPTER-IV (SECTION-B)

**Acid catalyzed green synthesis of novel pyrazolopyrimidineDerivatives** and **their biological evaluation.**

The pyrazolo-pyrimidine scaffold is a highly adaptable drug-like template, which is extensively used in the design of cellular apoptosis and cancer therapies. These compounds are capable to exert significant anticancer effects by the inhibition of different types of proteins, enzymes, and receptors which play crucial roles in cell division.Consequently, the synthesis and development of pyrazolo-fused derivatives have been emerged as an important heterocyclic system due to their broad area of biological activitiesas well as synthetic applications in medicinal chemistry.Particularly, pyrazolo[3,4-d]pyrimidine has been a fruitful source of inspiration for medicinal chemists for many years and attracted their attention due to their numerous activities.These derivatives exhibit a wide range of biological activities such as antitumor, anxiolytic and antimicrobial.

In addition, pyrazolo[1,5-*a*]pyrimidine compounds are widely used as inhibitors of cyclin-dependent kinases (CDKs), that are involved in mediating the transmission of mitogenic signals and numerous other cellular eventsincluding cell proliferation, differentiation, migration, metabolism and immune response. It is also observed that many of these derivatives may block proliferation of various cancer cell lines. Some of the selective CDK inhibitors (**XXIII** to **XXV**) have been described in the literature that is undergoing clinical trials (Figure).

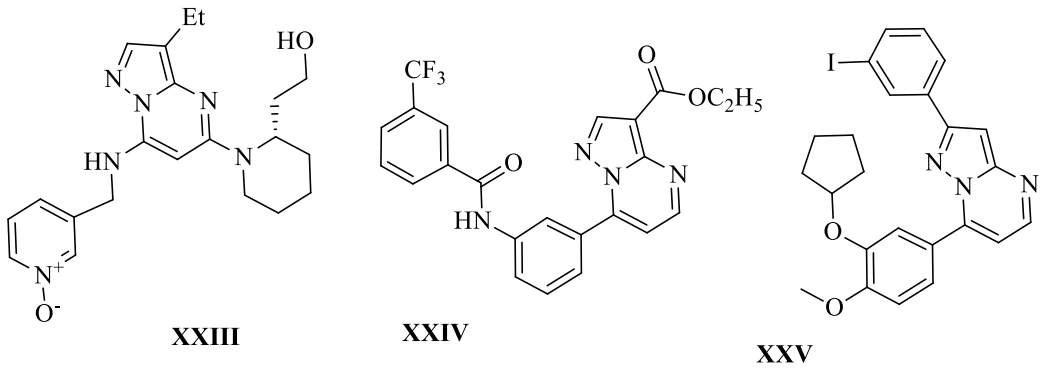
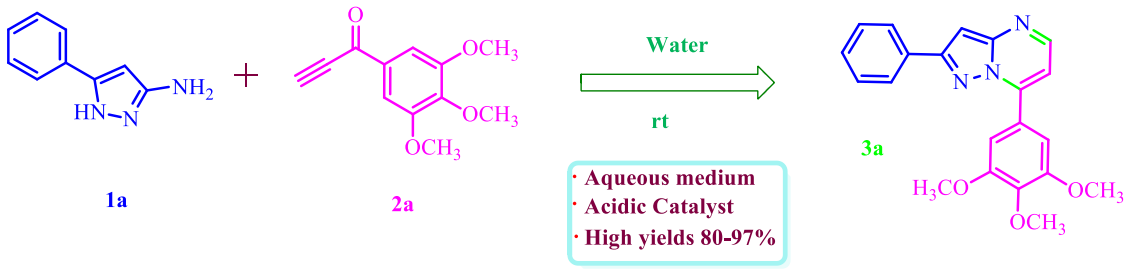



**Figure 8:** Chemical structures ofbiologically active pyrazolo[1,5-*a*]pyrimidine derivatives and CDK4 inhibitors

## Chemistry

Our studies began with exploring various non-catalytic routes for the cyclocondensation reaction between 1*H*-pyrazol-5-amine and keto-alkyne derivatives(Scheme 1).Some of the representative reaction conditions that were investigated,it was observed that this reaction did not proceed at room temperature when run without any catalyst in water, acetonitrile, methanol or ethanol. Interestingly, the product **3a** was obtained in 73% by adding the hydrochloric acid reaction at room temperature for 4 h. Subsequently it was revealed that the cyclocondensation proceeds efficiently in all the solvents tested under acidic conditions. Though PTSA in water and ethanol afforded high yields of products,as well, by the completion of reaction just filtration of the crude mixture obtained a pure product. Considering the operational simplicity water was preferred over other as the solvent.




**Scheme 7:** Green synthesis of pyrazolo-pyrimidinederivatives

In conclusion, a simple, mild, efficient and environmentally benign method for the synthesis of pyrazolo[1,5-a]pyrimidine scaffold has been developed by using acid

catalysts in water. The advantages of this method include its simplicity of operation, cleaner reactions, absence of side products and higher yields. Further, the purification of the product is simple, involving a simple filtration and washing.

(Manuscript prepared for ***RSC. Adv.)***